

**CLAIMS:**

- 5        1. An enriched preparation of human undifferentiated embryonic stem cells wherein said cells are capable of proliferation *in vitro* and differentiation to neural progenitor cells, neuron cells or glial cells.
- 10      2. The enriched preparation of human undifferentiated embryonic stem cells according to claim 1 wherein said cells maintain an undifferentiated state when cultured on a fibroblast feeder layer in the absence of a differentiating signal.
- 15      3. The enriched preparation of human undifferentiated embryonic stem cells according to claim 1 or 2 wherein said cells are capable of differentiation into neural progenitor cells.
- 20      4. An undifferentiated human embryonic stem cell wherein the cell is capable of proliferation *in vitro* and differentiation to neural progenitor cells, neuron cells or glial cells and is immunoreactive with markers for human pluripotent stem cells including SSEA-4, GCTM-2 antigen, and TRA 1-60.
- 25      5. The undifferentiated human embryonic stem cell according to claim 4 wherein the cell expresses Oct-4.
- 30      6. The undifferentiated human embryonic stem cell according to claim 5 wherein said cell maintains a diploid karyotype during prolonged cultivation *in vivo*.
- 35      7. The undifferentiated human embryonic stem cell according to claim 6 which forms tumors when injected in the testis of immunodeprived SCID mice.

TECHNICAL FIELD

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8. A differentiated committed human progenitor cell line capable of differentiation and propagation into mature neurons or glial cells said cell line derived from undifferentiated human embryonic stem cells.

9. The differentiated committed human progenitor cell line according to claim 8 capable of establishing a graft in a recipient brain.

10. The differentiated committed human progenitor cell line according to claim 9 capable of differentiating *in vivo* into other cell lineages including neurons and glial cells such as astrocytes and oligodendrocytes.

11. A neural progenitor cell differentiated *in vitro* from an undifferentiated human embryonic stem cell.

12. The neural progenitor cell according to claim 11 wherein said cell is capable of proliferation.

13. The neural progenitor cell according to claim 11 wherein said cell is capable of differentiating to a mature neuron cell or glial cell.

14. The neural progenitor cell according to claim 11 wherein said cell is capable of transdifferentiation into other cell lineages to generate stem cells and differentiated cells of non-neural phenotype.

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15. The differentiated neural progenitor cell according to claim 8 or 11 characterised by expressed markers including markers of the neuroectodermal lineage; markers of neural progenitor cells; neuro-filament proteins; monoclonal antibodies including MAP2ab; glutamate; synaptophysin; glutamic acid decarboxylase; tyrosine hydroxylase;  $\beta$ -tubulin;  $\beta$ -tubulin III; GABA  $\text{A}\alpha 2$  receptor, glial fibrillary acidic

protein (GFAP), galactocerebroside (gal C), 2', 3'- cyclic nucleotide 3'- phosphodiesterase (CNPase), *plp*, DM-20 and O4.

5        16. The neural progenitor cell according to claim 15 which expresses markers of neuroectoderm and neural progenitor cells selected from the group including NCAM, nestin, vimentin and the transcriptional factor Pax-6, and do not express Oct-4.

10      17. The neural progenitor cell according to claim 16 wherein said cell is capable of establishing a graft in a recipient brain.

18. The neural progenitor cell according to claim 17 wherein said cell can incorporate extensively into a recipient brain.

19. The neural progenitor cell according to claim 18 wherein said cell is capable of migrating along host brain pathways.

20. The neural progenitor cell according to claim 19 wherein said cell is responsive to host environmental signals.

21. The neural progenitor cell according to claim 20 wherein said cell differentiates in response to local host environmental signals.

22. The neural progenitor cell according to claim 20 wherein said cell is capable of differentiation to other cell lineages in a recipient brain.

25      23. The enriched preparation of neural progenitor cells including an enriched population of cells according to claim 15.

30      24. The enriched preparation of neural progenitor cells according to claim 23 wherein said cells are capable of prolonged undifferentiated proliferation.

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25. The enriched preparation of neural progenitor cells according to claim 23 wherein said cells are capable of differentiation into neurons, mature neurons and glial cells.

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26. The enriched preparation of neural progenitor cells according to claim 25 wherein said cells are capable of establishing a graft in a recipient brain in the absence of tumors.

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27. The enriched preparation of neural progenitor cells according to claim 26 wherein said cells may be recovered from cryopreservation.

28. A method of preparing undifferentiated human embryonic stem cells for differentiation into neural progenitor cells, said method including:

obtaining an *in vitro* fertilised human embryo and growing the embryo to a blastocyst stage of development;

removing inner cells mass (ICM) cells from the embryo;

culturing ICM cells under conditions which do not induce extraembryonic differentiation and cell death, and promote proliferation of undifferentiated stem cells;  
and

recovering stem cells.

29. The method according to claim 28 including culturing the ICM cells on a fibroblast feeder layer to promote proliferation of embryonic stem cells prior to recovering the stem cells from the feeder layer.

30. The method according to claim 29 wherein said fibroblasts are either human or mouse fibroblasts or a combination of human and mouse fibroblasts.

31. The method according to claim 30 wherein the fibroblast feeder layer comprises embryonic fibroblasts.

5 32. The method according to claim 31 wherein said fibroblasts are derived from inbred 129/Sv or CBA mice or mice from a cross of 129/Sv with C57/B16 strains.

10 33. The method according to claim 32 wherein said fibroblast feeder layer has a density of approximately 25,000 human and 70,000 mouse cells per cm<sup>2</sup> or 75,000 to 100,000 mouse cells per cm<sup>2</sup>.

34. The method according to claim 33 wherein the fibroblast feeder layer is established 6 to 48 hours prior to addition of ES or ICM cells.

15 35. The method according to any one of claim 34 wherein the fibroblast feeder cells are arrested in their growth.

36. The method according to claim 35 wherein the fibroblast feeder cells are arrested by irradiation or treated with mitomycin C.

20 37. The method according to claim 36 further including:  
replacing the stem cells from the fibroblast feeder layer onto another fibroblast feeder layer; and

culturing the stem cells for a period sufficient to promote proliferation of morphologically undifferentiated stem cells.

25 38. The undifferentiated human embryonic stem cell prepared by a method according to any one of claims 28 or 37.

30 39. A method of inducing somatic differentiation of stem cells *in vitro* into progenitor cells said method comprising:

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obtaining undifferentiated embryonic stem cells; and  
providing a differentiating signal under conditions which are non-permissive  
for stem cell renewal, do not kill cells and/or induces unidirectional differentiation  
toward extraembryonic lineages.

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40. The method according to claim 39 wherein said undifferentiated embryonic  
stem cell is capable of proliferation *in vitro* and differentiation to neural progenitor  
cells, neuron cells or glial cells and is immunoreactive with markers for human  
pluripotent stem cells including SSEA-4, GCTM-2 antigen, and TRA 1-60.

41. The method according to claim 39 wherein said undifferentiated embryonic  
stem cell expresses Oct-4.

42. The method according to claim 39 wherein said undifferentiated embryonic  
stem cell maintains a diploid karyotype during prolonged cultivation *in vivo*.

43. The method according to claim 39 wherein said undifferentiated embryonic  
stem cell forms tumors when injected in the testis of immunodeprived SCID mice.

44. The method according to claim 39 wherein said undifferentiated embryonic  
stem cell is prepared according to the method of claim 28.

45. The method according to claim 39 wherein said undifferentiated embryonic  
stem cell is prepared according to the method of claim 37.

46. The method according to claim 39 wherein the conditions for inducing somatic  
differentiation of stem cells are selected from any one of the following including:

culturing the undifferentiated stem cells for prolonged periods and at high  
density on a fibroblast feeder cell layer to induce differentiation;

culturing the undifferentiated stem cells in serum free media;

culturing the undifferentiated stem cells on a differentiation inducing fibroblast  
feeder layer and wherein said fibroblast feeder layer does not induce extra embryonic  
differentiation and cell death;

culturing to a high density in monolayer or on semi-permeable membranes so  
as to create structures mimicing the postimplantation phase of human development; or

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culturing in the presence of a chemical differentiation factor selected from the group including bone morphogenic protein-2 or antagonists thereof.

47. The differentiated progenitor cell prepared by the method according to claim

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48. The differentiated progenitor cell according to claim 47 selected from the group including a neural progenitor cell or mesodermal progenitor cell including hemangioblast or hematopoietic stem cells.

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49. The differentiated progenitor cell according to claim 48 which is a neural progenitor cell capable of differentiating into a neuron cell or a glial cell.

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50. A method of inducing somatic cells from embryonic stem cell derived somatic progenitors, said method comprising:

obtaining a source of embryonic stem cell derived somatic progenitors;  
culturing the progenitor cells on an adhesive substrate; and

inducing the cells to differentiate to somatic cells under conditions which favour somatic differentiation.

51. The method according to claim 50 wherein said embryonic stem cell derived somatic progenitor cells are grown in the presence of a serum free media and growth factors and are induced to differentiate by withdrawal of the growth factors.

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52. The method according to claim 50 wherein the embryonic stem cell-derived progenitor cell is prepared according to the method of claim 39.

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53. The method according to claim 50 wherein the embryonic stem cell-derived progenitor cell is selected from the group consisting of a neural progenitor cell or mesodermal progenitor cell including hemangioblast or hematopoietic stem cells.

54. The method according to claim 50 wherein the embryonic stem cell-derived progenitor cell is a neural progenitor cell capable of differentiating into a neuron cell or a glial cell.

55. The method according to claim 54 wherein the progenitor cells are cultured on an adhesive substrate selected from poly-D-lysine and laminin or poly-D-lysine and fibronectin.

56. The method according to claim 55 wherein the progenitor cells are cultured on poly-D-lysine and laminin.

57. The method according to claim 56 wherein the cells are further cultured in the presence of retinoic acid.

58. The method according to any one of claims 55 to 57 wherein said somatic cells induced are neurons including mature neurons.

59. The method according to claim 55 wherein the progenitor cells are cultured on poly-D-lysine and fibronectin.

60. The method according to claim 59 wherein the progenitor cells are cultured before and after plating on poly-D-lysine and fibronectin in serum free medium in the presence of PDGF-AA and bFGF.

61. The method according to claim 60 wherein the progenitor cells are cultured after plating in the presence of PDGF-AA, basic FGF and EGF .

62. The method according to claim 61 further including culturing the somatic progenitor cells after plating in the presence of T3.

63. The method according to claim 62 wherein said somatic cells induced are glial cells including astrocyte and oligodendrocyte cells.

5 64. A method of producing an enriched preparation of human ES derived neural progenitor cells, said method comprising:

obtaining an undifferentiated human embryonic stem cell comprising obtaining an *in vitro* fertilised human embryo and growing the embryo to a blastocyst stage of development;

removing inner cells mass (ICM) cells from the embryo;

10 culturing ICM cells under conditions which do not induce extraembryonic differentiation and cell death, and promote proliferation of undifferentiated stem cells; recovering stem cells;

inducing somatic differentiation of the embryonic stem cell to a neural progenitor cell comprising obtaining undifferentiated embryonic stem cells;

providing a differentiating signal under conditions which are non-permissive for stem cell renewal, do not kill cells or induces unidirectional differentiation toward extraembryonic lineages;

identifying a neural progenitor cell by expressed markers of primitive neuroectoderm and neural stem cells such as polysialylated N-CAM, intermediate filament proteins such as nestin and vimentin and the transcription factor Pax-6; and

culturing the neural progenitor cells to promote proliferation and propagation.

25 65. The method according to claim 64 wherein the neural progenitor cells are cultured as spheres or monolayers in serum free medium comprising DMEM/F12 supplemented with growth factors.

66. The method according to claim 65 wherein the growth factors include B27, EGF and bFGF.

67. The method according to claim 66 including further culturing to eliminate non-neural cells, said culturing comprising further selective culturing in serum free media including DMEM/F12 supplemented with growth factors.

5 68. The method according to claim 67 wherein the further culturing includes the transfer of undifferentiated ES cell clumps into serum free medium comprised of DMEM/F12 supplemented with B27, bFGF and EGF and cultivation of the resulting neural progenitors as spheres or monolayers.

10 69. A method of transplanting ES derived neural progenitor spheres, said method comprising:

disaggregating the spheres; and  
injecting the disaggregated spheres into a living host.

70. The method according to claim 69 wherein the ES derived neural progenitor spheres include ES-derived neural progenitor cells prepared by the method comprising obtaining undifferentiated embryonic stem cells; and  
providing a differentiating signal under conditions which are non-permissive for stem cell renewal, do not kill cells and/or induces unidirectional differentiation toward extraembryonic lineages.

71. The method according to claim 69 wherein disaggregation includes mechanical tituration

25 72. The method according to claim 71 wherein disaggregation includes digestion of the spheres with papain combined with mechanical tituration.

73. The method according to claim 69 wherein the disaggregated spheres are injected into the nervous system of a host.

74. The method according to claim 73 wherein the spheres are injected into the lateral cerebral ventricle.

5 75. A method for inducing somatic cells *in vivo* from embryonic stem cell derived somatic precursors, said method comprising:

10 obtaining a source of embryonic stem cell derived somatic progenitor cells prepared by a method comprising obtaining undifferentiated embryonic stem cells; providing a differentiating signal under conditions which are non-permissive for stem cell renewal, do not kill cells and/or induces unidirectional differentiation toward extraembryonic lineages; and

transplanting the somatic progenitors into a host to induce differentiation to somatic cells.

76. The method according to claim 75 wherein the method of transplanting the somatic progenitors comprises disaggregating the spheres; and

injecting the disaggregated spheres into a living host.

77. The method according to claim 75 wherein differentiation is induced by local host environmental signals.

78. A somatic cell prepared by a method according to claim 75.

79. A method of producing a stable graft of neural cells and contributing in the histogenesis of a living host said method comprising:

25 transplanting ES derived neural progenitor spheres into a living host according to a method comprising disaggregating the spheres; and

injecting the disaggregated spheres into a living host.

80. The method of modifying a nervous system of a host, said method comprising  
30 producing a stable graft of neural cells according to the method of claim 79.

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81. The method according to claim 80 wherein said modifying of the nervous system includes any one of replacing deficient neuronal or glial cell populations, restoring deficient functions or activating regenerative and healing processes in the nervous system.

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82. The method according to claim 81 wherein the neural progenitor spheres comprises genetically modified neural progenitor cells.

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83. The method according to claim 82 wherein the genetically modified neural progenitor cells express specific desired genes at the target organ.

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84. The method for treating a pathological condition of the nervous system comprising modifying a nervous system of a patient according to claim 80.

85. The method according to claim 84 wherein the pathological condition is selected from the group including neurodegenerative disorders, vascular conditions, autoimmune disorders, congenital disorders, and trauma.